Section II. REMARKS

(I). Request for A Two-Month Extension of Time

A two-month extension of time is hereby requested under the provisions of 37 CFR §1.136(a), which extends the deadline for responding to the October 1, 2003 Office Action to March 1, 2004.

The Office is hereby authorized to charge the $210.00 fee payable for the requested extension of time to the credit card specified in the Credit Card Payment Form enclosed herewith.

(II). Acknowledgement of Withdrawal of Claim Rejections

The Applicant hereby acknowledges the Examiner's withdrawal of previous rejections of claims 1-8, 17-19, 25-26, 31, 72, and 76-77 under 35 U.S.C. §112, second paragraph and of claims 1, 4, 6, 21, 25-28, and 31 under 35 U.S.C. §102(b) in the October 1, 2003 Office Action.

(III). Response to Rejections of Claims 1, 8, 71, 74, and 75

In the October 1, 2003 Office Action, the Examiner rejected claims 1, 8, 71, 74, and 75 for informalities under 35 U.S.C. §112, second paragraph.

Specifically, the Examiner rejected claim 1 for lack of clarity in the recited limitation "wherein the insoluble phase of the fusion protein is of sufficient mass to be centrifugally removed from a solution" therein (see Office Action, page 4, first and second paragraphs).

In response, Applicant has herein amended claim 1 by deleting such rejected claim limitation, and claim 1 as amended therefore overcomes the Examiner's rejection.

Further, claim 1 has been amended to require that "**said phase transition protein(s) has a molecular weight of at least 9,000 Daltons**," the support for which is provided on page 16, lines 11-17 of the instant specification.

In the event that the phase transition protein (e.g., the ELP tag) incorporated in a fusion protein is sufficiently long with a sufficiently large molecular weight (e.g., 9,000 Daltons), such fusion

protein, when undergoing phase transition, is capable of forming relatively large aggregates, which can be effectively retrieved by moderate centrifugation at normal solution conditions. In contrast, when the phase transition protein is truncated in length with a relatively small molecular weight (e.g., less than 9,000 Daltons), the solution conditions must be carefully selected and modified in order to effectuate formation of sufficiently large aggregates that can be retrieved by moderate centrifugation; otherwise, higher centrifugation power is required for retrieval of the fusion protein aggregates.

Therefore, by requiring phase transition proteins having molecular weights of at least 9,000 Daltons, the amended claim 1 of the present application unequivocally delineates and claims a group of fusion proteins that can be easily retrieved from the protein solution by inverse phase cycling under moderate centrifugation force, in compliance with the clarity or definiteness requirements of 35 U.S.C. 112, second paragraph.

Claims 8 and 71 have been amended herein to overcome the Examiner's rejections of such claims in the October 1, 2003 Office Action.

Further, claims 74 and 75 have been hereby amended to further clarify the relationship between the cleavage agent and the ELP fusion protein recited therein, and the amended claims 74 and 75 overcome the Examiner's rejections in the October 1, 2003 Office Action.

(IV). Response to Written Description Rejections

(A) Written Description for Fusion Proteins Exhibiting Phase Transition

The Examiner in the October 1, 2003 Office Action rejected claims 1, 3-10, 12-19, 21-28, 31-32, 66-77, and 78-72 for lack of written description under 35 U.S.C. §112, first paragraph, on the grounds that such claims "are directed to [a] large and variable genus of fusion proteins exhibiting a phase transition" (see Office Action, page 5, lines 13-14) and that the specification discloses only limited species within the claimed genus.

In response, Applicant has:

(1) appended in **Appendix II** hereof an Affidavit under 37 CFR §1.132 of Dr. Susan Dagher, presenting experimental data and results showing formation of thirty-six (36) different

fusion proteins that exhibit inverse phase transition behavior, while such fusion proteins comprise eleven (11) different target proteins joined to five (5) different phase transition proteins by eleven (11) different spacer peptides;

(2) amended claims 27 (from which claims 28 and 78 depend), 31 (from which claim 32 depends), 71 (from which claims 72-75 depend), and 82 herein, to recite the limitation "wherein said phase transition protein(s) comprises ... repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue," for further delineating a specific group of fusion proteins that contain polypentapeptides of basic repeating unit Val-Pro-Gly-X-Gly as the phase transition proteins;

(3) amended claim 82 to require "one or more biological molecules selected from the group consisting of superoxide dismutase, interferon, asparaginease, glutamase, arginase, arginine deaminase, adenosine deaminase ribonuclease, trypsin, chromotrypsin, papin, insulin, calcitonin, adrenocorticotropic hormone (ACTH), glucagon, somatosin, somatropin, somatomedin, parathyroid hormone, erthyropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, and vasopressin," for further delineating a specific group of fusion proteins that contain the listed target proteins; and

(4) provided in the ensuing discussion an element-by-element analysis of Applicant's claimed fusion proteins, in relation to the corresponding description of such claimed fusion proteins in the originally filed specification, the disclosed correlation between structures and functions of such claimed fusion proteins, the actual structure-function correlation observed during experimentation, the guidance provided by the originally filed specification for making the claimed fusion proteins, and the level of skill and knowledge in the field of art, which shows that the facts compel the conclusion of Applicant's actual possession of the claimed invention at the time the present application was filed and the patentability of the instant claims under 35 U.S.C. §112, first paragraph, as properly described by the originally filed specification.

Amended claim 1 of the present application, from which claims 3-10, 12-19, 21-26, 66-70, 76, 79, and 80 depend, recites:

"A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides and proteins;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition protein(s) of (b) and wherein said phase transition protein(s) has a molecular weight of at least 9,000 Daltons."

Independent claims 27, 31, 71, 81, and 82, from which claims 28, 32, 72-75, and 78 respective depend, contain corresponding limitations.

In the October 1, 2003 Office Action, the Examiner asserted that the instant specification fails to support the above-recited elements (a)-(c) with sufficient disclosure or teachings and that Applicant had no possession of the claimed invention at the time the present application was file.

Applicant vigorously disagrees, for the following reasons:

(1) Recited Element (a) and Written Description Thereof

Element (a) as recited by claims of the present application is a target protein of interest, which is joined with a phase transition protein (i.e., an ELP carrier) to form Applicant's claimed fusion protein.

A very important discovery made by the Applicant in the present invention is that a fusion protein formed by joining an ELP carrier exhibiting inverse phase transition with the target protein of interest "retains the inverse transition behavior of the ELP carrier" (see page 5, lines 18-19 of the instant specification).

Inclusion of the ELP component in the fusion protein ensures the inverse phase transition behavior of the fusion protein, and the size and composition of such ELP components further determine the transition temperature and yield of the fusion protein as well as the size of aggregates formed by such fusion protein during phase transition (see page 13-16, Section 5.1 of

the instant specification).

On the other hand, the target protein of interest does not ensure the phase transition of the fusion protein, and variations in the structure (including primary, secondary, and tertiary structures), molecular weight, electric charge, viscosity, and biological function of the target protein of interest do not prohibit phase transition of the fusion protein.

This is an **unexpected and surprising** advantage of Applicant's claimed fusion proteins, which enables broad applications of the present invention in isolating and purifying various target proteins of different physical and biological characteristics.

In the specification as originally filed, Applicant has demonstrated that the target protein of interest can be a soluble, overexpressed protein (e.g., the thioredoxin), or alternatively an insoluble, under-expressed protein (e.g., tendamistat). **The fusion protein incorporating either one of these two target proteins exhibits proper inverse phase transition behavior, regardless of the fundamental differences in solubility (i.e., hydrophobicity) and expression levels** of such target proteins (see instant specification, page 17, second and third paragraph).

However, the Examiner in the October 1, 2003 Office Action still rejected the specification for insufficient disclosure, on the basis that the specification does not "particularly point out which features of said [target] proteins ensure the capacity of exhibiting the phase transition of the fused protein" and does not "disclose any limitation on the structure of [target] proteins that may be used instead of thioredoxin and tendamistat" (see October 1, 2003 Office Action). Further, the Examiner stated that thioredoxin and tendamistat "cannot be considered representative number of species" of the large genus of target proteins, because "they do not allow for identification of the genus by basic biochemical chemical characteristics such as: 1) primary, secondary and tertiary structure, 2) molecular weigh[t], 3) electrical charge, 4) viscosity, 5) biological function" (see the Office Action, page 6, last paragraph).

In rebuttal of the Examiner's rejection, Applicant hereby encloses in **Appendix II** an Affidavit signed and sworn to by Dr. Susan Dagher, who is the primary research investigator of Phase BioScience, Inc. (Chapel Hill, North Carolina), the licensee of the present application, attesting to empirical work conducted by her involving uses of various target protein species.

Specifically, the Affidavit sets forth experimental data and results evidencing formation of thirty-six (36) different fusion proteins exhibiting inverse phase transition behavior, while such fusion proteins contain **eleven (11) different target proteins** joined with ELP carriers.

The eleven target proteins include:

- Insulin A peptide;
- T20 peptide;
- Interferon alpha 2B peptide;
- Tobacco etch virus protease;
- Small Heterodimer partner orphan receptor;
- Androgen receptor ligand binding domain;
- Glucocorticoid receptor ligand binding domain;
- Estrogen receptor ligand binding domain;
- G protein alpha Q;
- 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide; and
- G protein alpha S.

These target proteins have significantly different primary structures (i.e., the amino acid sequences), as shown in Section (A) of **Appendix B** of the Affidavit. The number of amino acid residues included in such target proteins ranges from as small as 21 to as large as 400, as indicated in **Appendix A** of the Affidavit.

The secondary and tertiary structures of these target proteins are fundamentally different. The target proteins as properly folded comprise different numbers of loops, α-helices, and β-sheets of different lengths, as arranged in different configurations. For example, the insulin A peptide contains a single loop structure, while the 1-Deoxy-D-Xylulos 5-Phosphate reductoisomerase peptide comprises as many as 13 α-helices and 11 β-sheets with numerous loops herebetween; the interferon alpha 2B peptide comprises a barrel-like 3-D structure formed by 6 α-helices linked together by loops, while the tobacco etch virus protease includes 2 α-helices and 10 β-sheets bundled together into a nearly cubic structure (see the protein structure database provided by NCBI at http://www.ncbi.nlm.nih.gov/entrez/ query.fcgi?db=Structure, visited on February 25, 2004).

The molecular weights of these target proteins are significantly different, ranging from as small as about 2.4 KDa for insulin A peptide to as large as 44.2 KDa for G protein alpha S, as shown in **Appendix A** of the Affidavit.

Further, **such target proteins are different in their electrical charge distributions and viscosity**, as stated in page 6-7 of the Affidavit and consistent with the corresponding differences in their compositions, sizes, and structures as shown hereinabove.

Finally, **the biological functions performed by such target proteins are fundamentally different**. For example, insulin A peptide functions to facilitate regulation of the metabolism of carbohydrates and fats, especially the conversion of glucose to glycogen, which lowers the blood glucose level; the T20 peptide is part of the T20 fusion inhibitor that blocks the HIV virus from merging or fusing with a cell before it can cause infection; the tobacco etch virus protease is a cysteine protease that cuts peptide bonds at specific cleavage sites; the 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide is part of an enzyme that catalyzes a pathway-specific step of the non-mevalonate pathway in cyanobacteria, etc.

Regardless of such fundamental differences in the structures, molecular weights, electric charge distributions, viscosity, and biological functions of these eleven (11) target proteins, all of the thirty-six (36) fusion proteins incorporating such target proteins retain the inverse phase transition behavior of the ELPs, as attested under oath by Dr. Susan Dagher in the Affidavit enclosed herewith.

Such Affidavit thus provides a further corroboration of the broad applicability of Applicant's claimed invention to other target protein species besides thioredoxin and tendamistat, consistent with the general teachings of the present application, and the fact that one of ordinary skill in the art can, upon reading the instant specification, readily apply the procedure in the examples of the specification to form fusion proteins exhibiting phase transition by using other target protein species.

In the October 1, 2003 Office Action, the Examiner found Applicant's argument for broad applicability of the claimed invention to a large genus of target proteins unpersuasive, on the basis that "*it is doubtful that large proteins, with molecular weight higher than 100,000 Da,*

exhibiting a large positive charge, are good candidates for [the target protein recited in] part (a) of the claimed fusion protein" (see Office Action, page 7, first and second paragraphs).

When the Examiner challenges the correctness of a statement in the specification and/or Applicant's response, he or she bears the burden of supporting such challenge with sound basis, and it is improper for the Examiner to refute an explicit teaching of operability stated by the Applicant on mere speculation, without substantive reasons. *Ex parte Dunn and Mathis*, 181 USPQ 652, 653 (BdPatApp&Int 1974).

In the present case, the Examiner did not provide any reference in support of his doubt of the operability of large proteins; nor did the Examiner present any scientific basis for his speculation that incorporation of large proteins with positive charge will prohibit the fusion protein from exhibiting phase transition.

Applicant in the specification has amply illustrated **numerous species of target proteins which may be used** for forming fusion proteins that exhibit phase transition behavior (see the instant specification, pages 16-17, naming over **twenty (20) types of target proteins** to which the present invention is applicable, including superoxide dismutase, interferon, asparaginease, glutamase, arginase, arginine deaminase, adenosine deaminase ribonuclease, trypsin, chromotrypsin, papin, insulin, calcitonin, adrenocorticotropic hormone (ACTH), glucagon, somatosin, somatropin, somatomedin, parathyroid hormone, erthyropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, and vasopressin).

In addition to the above, Applicant has showed **actual use of fifteen (15) specific target proteins** for forming fusion proteins that exhibit phase transition behavior, which include thioredoxin, tendamistat, green fluorescent protein (GFP), blue fluorescent protein (BFP), insulin A peptide, T20 peptide, interferon alpha 2B peptide, tobacco etch virus protease, small Heterodimer partner orphan receptor, androgen receptor ligand binding domain, glucocorticoid receptor ligand binding domain, estrogen receptor ligand binding domain, G protein alpha Q, 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide, and G protein alpha S.

In *In re Sarett*, 140 USPQ 474 (CCPA 1964), the Court specifically held that "it is certainly not incumbent on an applicant who has made a broad... invention and supported it by an adequately

broad disclosure to demonstrate the operativeness of every substance falling within the scope of the broad claims to which he is entitled," and that "the research to do this would quite evidently be endless."

Therefore, Applicant has provided sufficient evidence in support of the claimed broad applicability of the claimed invention.

(2) Recited Element (b) and Written Description Thereof

Element (b) recited by claims of the present application is a phase transition protein, which is incorporated into Applicant's claimed fusion protein to induce inverse phase transition of such fusion protein.

In the October 1, 2003 Office Action, the Examiner objected to the instant specification for failing to "give sufficient structural description" of such recited phase transition protein and "identify the structure of all elastin-like polypeptides" (see page 7 of the Office Action).

It has been well established that **what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail, and is preferably omitted, by a patent specification**. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (CAFC 1986), citing *Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Company et al.*, 221 USPQ 481 (CAFC 1984).

The structures of various phase transition proteins exhibiting inverse phase transition were well known at the time the present application was filed, as evidenced by International Patent Application PCT/US96/05186 (published as WO96/32406 on October 17, 1996), and U.S. Patent. Nos. 4,783,523, 4,870,055 4,898,926, and 5,527,610, which are cited by the instant specification on page 13, lines 17-20 and page 14, page 13-14. Each of these references thoroughly describes the structures and sequences of different kinds of elastin-like polypeptides that exhibit inverse phase transition.

Citation of these references by the instant specification reasonably conveys to a person ordinarily skilled in the art that Applicant had possession of various kinds of phase transition proteins as well known in the art at the time the present application was filed,

<u>and the instant specification does not have to describe in detail the well-known structures of such phase transition proteins</u>.

Therefore, it is improper for the Examiner to reject the specification for lack of structural description for the well-known phase transition proteins.

Moreover, the instant specification expressly discloses the sequences and primary structures of a specific group of phase transition proteins containing repeats of base pentapeptide IPGXG, but the Examiner found the specification lacking sufficient description even for this specifically disclosed group of phase transition proteins, on the basis that the specification did not disclose that use of this group of phase transition proteins will have the same effect as that of VPGXG (see the Office Action, page 7, lines 16-18).

The Examiner's reasoning is fallacious. The written description requirements of 35 U.S.C. 112, first paragraph only require that a patent specification describe the claimed invention "in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention" (see the Revised Interim Guidelines for Examination of Patent Applications Under the 35 USC §112, "Written Description" Requirement, 64 Fed. Reg. 71427 (December 21, 1999)). **<u>The law, however, does not require in any manner that an Applicant must show in the specification that use of an expressly disclosed alternative species has the same effect or achieves the same result as that of a preferred species in order to satisfy the written description requirements</u>**, and it is impermissible for the Examiner to arbitrarily impose such requirement upon the Applicant.

Therefore, the Examiner's rejections of claims 1, 3-10, 27-28, 31-32, 66-77, and 78-82 for lack of written description are improper.

Despite the improper claim rejections made by the Examiner, Applicant has hereby amended claims 27 (from which claims 28 and 78 depend), 31 (from which claim 32 depends), 71 (from which claims 72-75 depend), and 82, by adding a new claim limitation "wherein said phase transition protein(s) comprises ... repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue." Such new limitation clearly delineates a specific group of fusion proteins that contain polypentapeptides of basic repeating unit Val-Pro-Gly-X-Gly as the phase transition proteins, the production, isolation, and purification of which are

described in great detail by both the instant specification and the Affidavit of Dr. Susan Dagher.

(3) Recited Element (c) and Written Description Thereof

Element (c) recited by claims of the present application is a spacer peptide for separating the target protein and the phase transition protein.

In the October 1, 2004 Office Action, the Examiner objected to the recitation of such spacer peptide in the claims of the present invention, on the basis that the specification lacks identification of structural characteristics of such spacer peptide.

However, since the primary function of such spacer peptide is to separate the target protein and the phase transition protein in the fusion protein containing same, it is not limited to any specific sequence or structure. In other words, any peptide of any primary, secondary, or tertiary structure can be used as the spacer peptide to be incorporated in the claimed fusion protein, and the structural characteristics of such spacer peptide are neither essential nor critical to Applicant's claimed invention.[2]

Therefore, the Examiner's requirements for identification of structural characteristics of such spacer peptide in the specification are not supported by substantive reasons.

(B) Written Description for Mechanisms Useful for Inducing Phase Transition

In the October 1, 2003 Office Action, the Examiner rejected claims 9, 66-72, and 74-75 on the basis that "neither the claim nor the specification describe[s] the phase transition of the claimed fusion protein wherein the phase transition is mediated by changing pH, addition of solutes and/or solvents, side chain ionization, chemical modification, and changing pressure" (see Office Action, page 8, lines 9-12).

First, the Examiner's basis of rejection is not supported by the facts of this case.

[2] Although in a preferred embodiment of the present invention, the spacer peptide comprises a cleavage site recognizable by a protease, which enables subsequent cleavage of the fusion protein containing same, such spacer peptide may not include any cleavage site, if subsequent cleavage of the fusion protein is not desired or needed.

Induction of phase transition by addition of solutes, such as NaCl, is described in detail on page 15, lines 18-19, page 21, lines 7-11, page 37, lines 8-20, page 38, lines 11-25 of the instant specification. For example, the instant specification discloses on page 4, lines 9-12 that the inverse phase transition of an ELP fusion protein can be triggered by "depressing the [transition temperature] T_t below the solution temperature by the addition of NaCl to the solution," while the solution temperature maintains the same.

Therefore, the Examiner's assertion that "neither the claim nor the specification describe[s] the phase transition of the claimed fusion protein wherein the phase transition is mediated by... addition of solutes" is incorrect.

In the October 1, 2003 Office Action, the Examiner conceded that the instant specification "teaches how changes [in] NaCl concentration would change the transition temperature," but then asserted that "it is not clear how the change in NaCl itself would induce phase transition" (see Office Action, page 8, lines 14-16).

However, the instant specification has disclosed that inverse phase transition occurs when the solution temperature is equal to or higher than the transition temperature of a phase transition protein (see instant specification, page 2, lines 12-15). Logic follows that such phase transition can thus be triggered either by increasing the solution temperature, or by decreasing the transition temperature. Since increase in NaCl concentration causes decrease in the transition temperature, as shown in Figure 6 of the instant specification, the phase transition can be induced by adding sufficient NaCl into the solution, to increase the NaCl concentration in the solution and cause decrease of the transition temperature to below the solution temperature. A person ordinarily skilled in the art, upon reading the instant specification, therefore will readily understand how the change in NaCl concentration in the solution induces the phase transition of the fusion protein therein.

Further, as mentioned hereinabove, the instant specification has expressly stated on page 4, lines 9-12 that the inverse phase transition of an ELP fusion protein can be triggered by "depressing the [transition temperature] T_t below the solution temperature by the addition of NaCl to the solution."

Applicant therefore fails to apprehend the reason why "it is not clear how the change in NaCl...

would induce phase transition," as asserted by the Examiner, and further clarification is respectfully requested.

Secondly, as mentioned hereinabove, it has been well established that **what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail, and is preferably omitted, by a patent specification**. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (CAFC 1986), citing *Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Company et al.*, 221 USPQ 481 (CAFC 1984).

Mechanisms that can be used for modulating the transition temperature to induce inverse phase transition were well known at the time the present application was filed.

For example, D.W. Urry, *Free Energy Transduction in Polypeptides and Proteins Based on Inverse Temperature Transitions*, PROG. BIOPHYS. MOLEC. BIOL., vol. 57, pp 23-57 (1992) (hereinafter "Urry"), as submitted to the Office with the June 29, 2001 Information Disclosure Statement, describes in detail various mechanisms that may be used for modulating the transition temperature of a phase transition protein. Specifically, it discloses that phase transition can be induced by a change in pH (see Urry, pages 36 and 39, and Figure 14); it also discloses the use of solvents, such as ethylene glycol, dimethyl sulfoxide and urea, for modulating the transition temperature T_t (see Urry, page 37); it further discloses that a change in external pressure results in a change in the transition temperature T_t when the phase transition protein comprises certain aromatic residues such as Trp, Phe, and Tyr (see Urry, page 37).

Therefore, the instant specification does not have to describe in detail each and every one of the phase transition induction mechanisms recited in claims 9, 66-72, and 74-75, because such mechanisms were well known in the art at the time the present application was filed.

The Examiner's rejections of claims 9, 66-72, and 74-75 for lack of written description are improper.

(V). Patentable Distinctions of Amended Claims 1, 27, 31, 71, 81, and 82 and Associated Dependent Claims Over McPherson

Claim 1 (from which claims 3-10, 12-19, 21-26, 66-70, 76, 79, and 80 depend) has been hereby

amended to recite a fusion protein that comprises **a phase transition protein having a molecular weight of at least 9,000 Daltons**." Claims 31 (from which claim 32 depend), 71 (from which claims 72 and 74-75 depend), and 81 have been amended to recite corresponding limitation. Further, claim 27 (from which claims 28 and 78 depend) has been amended to recite a fusion protein that comprises **a phase transition protein having at least thirty repeats of the pentapeptide Val-Pro-Gly-X-Gly**.

Amended claims 1, 27, 31, 71, and 81 and their associated dependent claims patentably distinguish over the previously cited reference, D.T. McPherson, C. Morrow, D.S. Minehan, J. Xu, E. Hunter, D.W. Urry, "Production and Purification of a Recombinant Elastomeric Polypeptide G-$(VPGVG)_{19}$-VPGV, from *Escherichia coli*," *Biotechnol. Prog.*, 8, 347-352 (1992) (hereinafter "McPherson"), for the following reaons:

The McPherson reference discloses a fusion protein that comprises a phase transition protein (G-$(VPGVG)_{19}$-VPGV) containing only 19 repeats of VPGVG, and the molecular weight of such phase transition protein disclosed by McPherson is only about 8,200 Daltons, which is less than 9,000 Daltons as required by the amended independent claims 1, 27, 31, 71, and 81 of the present application. More importantly, McPherson does not teach or suggest in any manner about forming fusion proteins that comprise phase transition proteins of more VPGVG repeats or larger molecular weight; nor does McPherson provide any motivation for a person ordinarily skilled in the art to modify the disclosed fusion protein, gst-G$(VPGVG)_{19}$-VPG.

McPherson therefore does not provide any derivative basis for Applicant's claimed invention, as recited in the amended independent claims 1, 27, 31, 71, and 81.

Further, claim 82 has been herein amended to recite a fusion protein that comprises "**one or more biological molecules selected from the group consisting of superoxide dismutase, interferon, asparaginease, glutamase, arginase, arginine deaminase, adenosine deaminase ribonuclease, trypsin, chromotrypsin, papin, insulin, calcitonin, adrenocorticotropic hormone (ACTH), glucagon, somatosin, somatropin, somatomedin, parathyroid hormone, erthyropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, and vasopressin**," as disclosed by the instant specification on pages 16-17.

The McPherson reference only discloses a fusion protein that comprises glutathione S-transferase

(gst) (see McPherson, page 347, right column, first full paragraph). McPherson does not teach or suggest in any manner about forming fusion proteins that comprise the biological molecules recited by claim 82 of the present application; nor does McPherson provide any motivation for a person ordinarily skilled in the art to modify the disclosed fusion protein, gst-G(VPGVG)$_{19}$-VPG, by substituting gst with another protein.

Therefore, the amended claim 82 patentably distinguishes over the McPherson reference.

(VI). Fees Payable

A Credit Card Payment Form is enclosed herewith, authorizing the Office to charge the $210.00 fee payable for two-month extension of time to the credit card identified therein.

Authorization hereby is given to charge any additional fee or to credit any overpayment to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.

CONCLUSION

Based on the amendments made herein and the foregoing remarks, pending claims 1, 3-10, 12-19, 21-28, 31-32, 62-72, 74-76 and 78-82 as amended herein are now in form and condition for allowance. The Examiner therefore is respectfully requested to reconsider and allow such amended claims.

Respectfully submitted,

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BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE
UNITED STATES PATENT AND TRADEMARK OFFICE

LIMITED RECOGNITION UNDER 37 CFR § 10.9(b)

Yongzhi Yang is hereby given limited recognition under 37 CFR § 10.9(b) as an employee of Intellectual Property Technology Law to prepare and prosecute patent applications wherein the patent applicant is the client of Intellectual Property Technology Law, and the attorney or agent of record in the applications is a registered practitioner who is a member of Intellectual Property Technology Law. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Yongzhi Yang ceases to lawfully reside in the United States, (ii) Yongzhi Yang's employment with Intellectual Property Technology Law ceases or is terminated, or (iii) Yongzhi Yang ceases to remain or reside in the United States, authorized to be employed by an Employment Authorization Card issued pursuant to 8 CFR § 274a.12(c)(9).

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the United States Patent and Trademark Office.

Expires: August 28, 2004

Harry I. Moatz
Director of Enrollment and Discipline

APPENDIX I

Clean Copy of All Pending Claims

1. A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides and proteins;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition protein(s) of (b) and wherein said phase transition protein(s) has a molecular weight of at least 9,000 Daltons.

3. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a peptide.

4. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a biologically active protein.

5. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a therapeutic protein.

6. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an enzyme useful in industrial biocatalysis.

7. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an antibody or antibody fragment.

8. The fusion protein of claim 7 wherein the antibody or antibody fragment has complex forming affinity for an antigenic protein of interest, and wherein upon binding to the antigenic protein of interest, the fusion protein retains ~~some or all of~~ its phase transition

character.

9. The fusion protein of claim 1 wherein the phase transition is mediated by one or more means selected from the group comprising:

changing temperature;

changing pH;

addition of solutes and/or solvents,

side-chain ionization or chemical modification; and

changing pressure.

10. The fusion protein of claim 1 wherein the phase transition is mediated by means comprising raising temperature.

12. The fusion protein of claim 1 wherein the one or more phase transition protein(s) of 1(b) comprises oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, wherein X is any natural or non-natural amino acid residue, and wherein X optionally varies among oligomeric repeats.

13. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a naturally-occurring amino acid residue.

14. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a non-naturally-occurring amino acid residue.

15. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) one or more amino acid residues selected from the group consisting of: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine residues.

16. The fusion protein of claim 12 wherein any two or more of the oligomeric repeats are separated by one or more amino acid residues which do not eliminate the phase transition characteristic of the fusion protein.

17. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) is greater than about 75%.

18. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) is greater than about 85%.

19. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the phase transition protein(s) of 1(b) is greater than about 95%.

21. The fusion protein of claim 1 wherein the spacer sequence comprises a proteolytic cleavage site.

22. The fusion protein of claim 1 wherein the fusion protein further comprises a signal peptide.

23. The fusion protein of claim 22 wherein the signal peptide is cleavable from the fusion protein by enzymatic cleavage.

24. The fusion protein of claim 22 wherein the signal peptide directs secretion of the fusion protein from the cell.

25. The fusion protein of claim 1 wherein the fusion protein is recombinantly produced.

26. The fusion protein of claim 1 wherein any of the biological molecule(s) of 1(a), phase transition protein(s) of 1(b), or spacer sequence of 1(c) (when present) is recombinantly produced.

27. A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides and proteins;

(b) one or more phase transition protein(s) that exhibit an inverse phase transition, wherein the one or more phase transition protein(s) are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecules of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), and wherein said phase transition protein(s) comprises at least thirty repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue.

28. The fusion protein of claim 27 wherein the phase transition is mediated by means comprising raising temperature.

31. A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides and proteins;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), wherein said phase transition protein(s) comprises oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue, and wherein said phase transition protein(s) has a molecular weight of at least 9,000 Daltons.

32. The fusion protein of claim 31 wherein the phase transition is mediated by raising temperature.

62. A method of optimizing size of an ELP expression tag incorporated in a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein the fusion protein comprises a protein of interest, said method comprising the steps of (i) forming a multiplicity of polynucleotides comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein each of said multiplicity

of polynucleotides includes a different-sized ELP expression tag, (ii) expressing corresponding fusion proteins from said multiplicity of polynucleotides, (iii) determining a yield of the desired protein for each of said corresponding fusion proteins, (iv) determining size of particulates for each of said corresponding fusion proteins in solution as temperature is raised above T_t, and (v) selecting an optimized size ELP expression tag according to predetermined selection criteria for maximum recoverable protein of interest from among said multiplicity of polynucleotides.

63. A method of purification of fusion proteins to yield a protein of interest, comprising forming a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, expressing the fusion protein in culture, and subjecting a fusion protein-containing material from said culture to processing involving centrifugation and inverse transition cycling to recover said protein of interest.

64. The method of claim 63, comprising expressing the fusion protein in culture in a well of a microplate.

65. The method of claim 63, comprising processing the fusion protein-containing material from said culture in a well of a microplate.

66. The fusion protein of claim 9, wherein the phase transition is mediated by addition of solute.

67. The fusion protein of claim 66, wherein the solute comprises an organic solute.

68. The fusion protein of claim 66, wherein the solute comprises an ionic solute.

69. The fusion protein of claim 68, wherein the ionic solute comprises a salt.

70. The fusion protein of claim 66, wherein the salt comprises NaCl.

71. An elastin-like polypeptide (ELP) fusion protein comprising a protein of interest and an elastin-like polypeptide component coupled by a cleavage site in a composition comprising a solvent medium in which the ELP fusion protein exhibits an inverse phase transition wherein the phase transition is mediated by at least one change selected from

the group consisting of:

(a) changing temperature;

(b) changing pH;

(c) addition of solutes and/or solvents;

(d) side-chain ionization or chemical modification; and

(e) changing pressure,

wherein the phase transition protein(s) of (b) comprises oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue, and wherein said phase transition protein(s) has a molecular weight of at least 9,000 Daltons.

72. The ELP fusion protein of claim 71, wherein the protein of interest is cleavable from the elastin-like polypeptide component at the cleavage site to yield the protein of interest and the elastin-like polypeptide component as cleavage products.

74. The ELP fusion protein of claim 72, wherein the protein of interest is cleavable from the elastin-like polypeptide component at the cleavage site by a cleavage agent to yield the protein of interest and the elastin-like polypeptide component as cleavage products.

75. The ELP fusion protein of claim 74, wherein said cleavage agent is a proteolytic agent for proteolytically cleaving the cleavage site of the ELP fusion protein.

76. The fusion protein of claim 12 wherein the phase transition protein(s) comprise a β-turn structure.

78. The fusion protein of claim 27 wherein the phase transition temperature is from about 35 to about 60°C.

79. The fusion protein of claim 1 wherein the phase transition protein(s) of (b) are joined to the N-terminus of the biological molecule(s) of (a).

80. The fusion protein of claim 21 wherein the proteolytic cleavage site is cleavable by a protease agent selected from the group consisting of serine, cysteine, aspartyl and metallo-proteases.

81. A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of peptides, therapeutic proteins and antibodies or antibody fragments;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a); and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b), and wherein the phase transition protein(s) of (b) has a molecular weight of at least 9,000 Daltons.

82. A fusion protein comprising:

(a) one or more biological molecules selected from the group consisting of superoxide dismutase, interferon, asparaginease, glutamase, arginase, arginine deaminase, adenosine deaminase ribonuclease, trypsin, chromotrypsin, papin, insulin, calcitonin, adrenocorticotropic hormone (ACTH), glucagon, somatosin, somatropin, somatomedin, parathyroid hormone, erthyropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, and vasopressin;

(b) one or more phase transition proteins that exhibit an inverse phase transition, wherein the one or more phase transition proteins are joined to the biological molecule(s) of (a), and wherein said phase transition protein(s) comprises oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, in which X is any natural or non-natural amino acid residue; and

(c) optionally, a spacer sequence separating any of the phase transition protein(s) of (b) from any of the biological molecule(s) of (a),

wherein the fusion protein retains the inverse phase transition behavior of the phase transition proteins of (b).

83. A method of conducting biocatalysis including the steps of adding a biocatalytic enzyme to a solution to facilitate biocatalysis therein in production of a product, and isolating the enzyme from the solution to separate the enzyme from the product, wherein the enzyme comprises an enzyme-fusion protein (EFP), wherein the EFP comprises an ELP.

84. The method of claim 83, wherein the enzyme after separation is recycled for subsequent rounds of biocatalysis.

85. A biocatalysis process, comprising use of an enzyme-fusion protein including an ELP to facilitate said biocatalysis.